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BULLETIN No. 26. V. P. P. 79.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.
ALBERT F. WOODS, CHIEF.

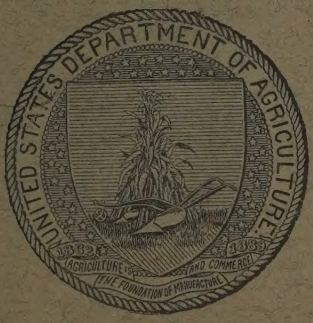
WAKKER'S HYACINTH GERM,

Pseudomonas hyacinthi (Wakker).

BY

ERWIN F. SMITH,
IN CHARGE OF LABORATORY OF PLANT PATHOLOGY.

Issued February 21, 1901.



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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY,
Washington, D. C., October 6, 1900.

SIR: I respectfully transmit herewith and recommend for publication a report by Dr. Erwin F. Smith, of this Division, on a bacterial disease of hyacinths commonly known as "the yellow disease" or "Wakker's disease."

The fact that large numbers of hyacinth bulbs are forced each year in the United States makes it desirable that their diseases be understood. The information gained regarding the biology of the organism will also be of great value to those investigating the bacterial diseases of plants.

The report confirms earlier work done in the Netherlands and adds much new and important information respecting the nature of the parasite. The latter belongs to a group of bacteria, hitherto but little studied, several members of which (also studied by Dr. Smith) cause diseases widely prevalent in the United States.

The report while primarily for pathologists and bacteriologists will also be of interest to florists and any others who wish to detect this disease and to avoid its introduction into the United States.

Respectfully,

ALBERT F. WOODS,
Chief of Division.

Hon. JAMES WILSON,
Secretary of Agriculture.

PREFACE.

This paper was prepared for publication in August, 1897, at which time I had secured characteristic infections and had worked out many of the cultural and other characters given in the following pages. The fact that I had not again produced the disease with germs isolated from my first series of infected plants, the further fact that I could not satisfactorily explain the meager growth of the parasite in the host plant, and on steamed potato and the other culture media which I had used, and, finally, a shadow of doubt concerning the accuracy of two or three other observations, induced me to withhold the paper and repeat the experiments. In the time which has intervened I have gone over nearly or quite all of the experiments detailed in the original paper, without, however, discovering any serious errors. During this time reinfections have been secured, the reason for the feeble parasitism has been discovered, and a number of other interesting facts have been brought to light, so that the long delay of publication has not been without its compensations.

Throughout this study numerous comparisons have been made with two other yellow bacteria, *Pseudomonas campestris* and *Ps. phaseoli*, and occasional mention has been made of them in this paper, both being plant parasites. Occasional comparisons have also been made with other bacteria, especially with *Ps. Stewarti*. The leading cultural characters of the hyacinth organism are mentioned in the synopsis at the end of this paper, but it has been decided to relegate an account of the numerous experiments on which these conclusions rest to a second bulletin, which is now ready for publication and in which they will be discussed in connection with the cultural peculiarities of the other yellow species of Pseudomonas here mentioned.

It is too much to hope that this bulletin is entirely free from mistakes. Nevertheless great pains have been taken to make it trustworthy, all of the experiments having been performed in duplicate, and nearly all of them having been repeated several times on different occasions to eliminate unsuspected sources of error.

Some brief statements respecting the morphology and physiology of this organism, as determined by the writer, were made at the Detroit meeting of The American Association for the Advancement of Science in August, 1897, and were published in the Proceedings of the Association for that year (Vol. XLVI, 1897, Salem, June, 1898).

ERWIN F. SMITH.

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WAKKER'S HYACINTH GERM,

Pseudomonas hyacinthi (Wakker).

HISTORICAL.

Dr. J. H. Wakker published five papers on the hyacinth germ between 1883 and 1888. His studies attracted wide attention because he was one of the earliest investigators in the field of plant bacteriology in a time of general skepticism and uncertainty, and also because of the great care with which he seemed to have worked out his results. Since the conclusion of Dr. Wakker's studies, which were begun in 1880, no bacteriologist or plant pathologist seems to have given any personal attention to the disease. Several pathologists have written about it or referred to it,[1] but nothing of any value has been added, and some of the comments have served only to throw doubt on the original inquiry.

In reading Dr. Wakker's papers for the purpose of making an abstract, I was at once struck with the need of a reinvestigation of the subject. This seemed necessary for two reasons: (1) Methods of isolation were not then as well understood as at present, and most of Wakker's successful infections seem to have been direct ones; (2) the germ is so imperfectly described that, excluding the test of pathogenesis, the identification of any particular organism as *Bacterium hyacinthi* Wakker would be altogether impossible. No disparagement of Dr. Wakker's beautiful studies is here intended. At the same time nothing perhaps better serves to illustrate the important advances

[1] De Bary: Vorlesungen über Bacterien, Leipzig, 1885, p. 137: also 3. Auflage. Leipzig, 1900, p. 173.

Sorauer: Handbuch der Pflanzenkrankh., 2. Auflage. 2. Theil. Berlin, 1886, p. 99.

Kramer: Die Bakteriologie in ihren Beziehungen zur Landwirtschaft, etc. Erster Theil, Wien, 1890, p. 145.

Comes: Crittogamia Agraria, Napoli, 1891, p. 510.

Ludwig: Lehrbuch der niederen Kryptogamen. Stuttgart, 1892, p. 96.

Tubeuf: Pflanzenkr. durch kryptogame Parasiten verursacht. Berlin. 1895, p. 556.

Prillieux: Maladies des Plantes agricoles et des Arbres fruitiers et forestiers causées par des parasites végétaux. Paris. 1895. Tome I, p. 22.

Frank: Die Krankheiten der Pflanzen. 2. Auflage, 2. Band, Breslau, 1896, p. 23.

Migula: System der Baketrien, 1. Bd., Jena, 1897, p. 320.

Hartig: Lehrbuch der Pflanzenkrankheiten. 3. Auflage. Berlin. 1900, p. 209.

which have been made in the technique of bacteriology than a perusal of the best early papers.

It is not unlikely that the additions which I shall make will also be insufficient, exclusive of the pathogenic test, to differentiate this germ ten or twenty years hence, but they will at least help toward definitely settling the group to which it belongs. Readers who wish merely a summary of Dr. Wakker's conclusions will find it in my critical review already cited, and those who wish to read the original papers will find the necessary references in the same paper.[1] Inasmuch as that review is very full and readily accessible, I may be excused from going over the ground again in this place.

SOURCE OF MATERIAL.

The hyacinth bulbs from which the germ that I have studied was isolated were said to be in the first stages of the yellow disease, and were sent to me in October, 1896, by Messrs. Van Meerbeck & Co., growers of bulbs at Hillegom, near Haarlem, Netherlands. The bulbs were sound externally. They had been "visited,"[2] and some of the vascular bundles of the inner scales were yellow, broken down, gummy, and full of bacteria. Penicillium was also present in places. No difficulty was experienced in isolating a yellow micro-organism from the broken down bundles of one of these bulbs, and subsequently the same germ was isolated from another bulb of the same lot. By planting a third bulb the disease was also obtained the following year in a daughter bulb. I have now cultivated this organism over four years in hundreds of cultures on a great variety of media, and have also obtained very satisfactory infections—infections so exactly like those described by Dr. Wakker that there can be no doubt either as to the nature of the organism with which I have worked or as to the substantial accuracy of Dr. Wakker's conclusions respecting its pathogenic properties.

INOCULATIONS OF 1897.

SERIES 1 (HYACINTHS).

The first set of inoculations was made February 16, 1897, from a pure beef-broth culture. Eight vigorous hyacinths were inoculated. They were all of one variety, a robust, single-flowered, deep-blue sort (name unknown). The plants were just coming into blossom and were the picture of health, six of the eight bulbs being large and well-stocked with food, and the other two smaller daughter bulbs. Part of the inoculations were by means of ordinary needle punctures and

[1] The Bacterial Diseases of Plants: A Critical Review of the Present State of our Knowledge, Parts III and IV, *The American Naturalist*, October and November, 1896, pp. 797, 912.

[2] Removal of the top of the bulb with a sharp knife for purposes of inspection is called "visiting." This is done after the bulbs are dug.

the rest by means of a hypodermic syringe, the results being the same, except that the symptoms appeared sooner when a large number of germs were inserted. All of the inoculations were made in the middle or terminal parts of healthy leaves, with one exception, in which case the germs were inserted into the upper part of a flower shaft before the buds opened.

Much to my surprise, the progress of the disease was very slow, exactly as described by Dr. Wakker, and the striping down of the disease was restricted in most cases to long, narrow areas, with healthy green tissue to either side. In case of the hypodermic injections, however, a width of three to eight or more vascular bundles was involved, i. e., as much breadth of tissue as appeared water-soaked after the injection, but not much more. Even when a great quantity of germs was injected (0.5 cc. or more of a fluid culture) the disease did not appear immediately, develop rapidly, or cause widespread infection of the bulbs.

To show how closely my results tally with those obtained by Dr. Wakker,[1] I will here set down the course of the disease in each of the eight plants first inoculated.

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[1] See Contributions à la pathologie végétale, I, La maladie du jaune, ou maladie nouvelle des jacinthes, causée par le *Bacterium Hyacinthi*. *Archives néerlandaises des sci. ex. et naturelles*, Tome XXIII, pp. 18–20.

Table showing result of inoculations with a pure culture of Pseudomonas hyacinthi (Wakker).

No. of plant.	Date of inoculation, place, manner, and immediate result.	Result of subsequent examinations.					
	Feb. 16, 1897.	Mar. 1.	Mar. 8.	Mar. 13.	Mar. 22.	June 10.	June 23.
I.	First leaf, upper part. Hypodermic. The injection produced a suffused place 1.5 mm. wide and 5 cm. long.	A yellow streak with shrinkage of tissue—confined to the suffused area. Rest of leaf very green and healthy, except 3 small yellow spots where I made ineffectual attempts to insert the needle.	Cut away leaf about 3 inches above bulb and photographed.	No symptoms on base of leaf.	Same as on 13th.	No symptoms. Leaves of plant now dried out except bases, which are yellow-green.	Leaves dried out. Bulb dry and sound-looking. Cut open; sound internally, except one inner scale. One bundle of this scale is bright yellow and is disorganized, being full of a yellow bacterial slime. The disease extends in this one bundle the whole length of scale and spreads out in the plateau, penetrating nearly to base of bulb and covering therein nearly 1 sq. cm. of tissue. Other bundles not examined microscopically.
	Second leaf, upper part. 20 to 30 ordinary needle pricks over less than 1 sq. cm.	Pricked area pale yellow green.	No record.....	Tissue dead and thin. The veins are brownish and the parenchyma between is pale yellow or white diaphanous. Disease begins to stripe down a few cm. in one place.	Pricked part dead and yellow-brown. A narrow interrupted stripe extends down 3 cm. and appears again as a very small spot 1.5 cm. lower.		
	Third leaf, upper part. Like No. 2, i.e., 20 to 30 delicate needle pricks.	Pricked area pale yellow-green.	No record.....	Decided symptoms over a breadth of 5 mm. and a length of 3 cm., commencing with the pricked area. The punctured part is diaphanous, shriveled, with a feeble browning of the veins.	The terminal 3 cm. of leaf has collapsed and is yellowish-brown. A narrow stripe extends down 2 cm. into the green.		

II.	Leaf, middle part. 20 to 30 needle punctures.	Pricked area pale yellow-green.	No record.....	Plain symptoms in the pricked area, with a narrow stripe up and down.	Apex of leaf (4 cm.) brown, shriveled. Diseased stripe rather wide and at least 12 cm. long. All the middle part of the leaf is yellow. Margins green.	Leaves of plant have dried out from age.	Small bulb. Sound externally. Five vascular bundles are bright yellow, gummy, and broken down by bacteria. Four of the yellow bundles are in an outer scale and one is in the next inner scale. The yellow, gummy, bacterial disease extends in these bundles the whole length of the scales and widens out below in the plateau.[1] The rest of the scales are sound.
III.	First leaf, upper part. Hypodermic. The injection produced a water-soaked place 3 to 4 mm. wide and 10 cm. long, i. e., including a breadth of 6 to 7 vascular bundles.	Conspicuous infection. The whole of the suffused area is diseased. The upper part is shriveled, thin, yellowish, and brownish in streaks (in the bundles). The lower part has a water-soaked, pale look, as if it were still suffused from the injection, which is not the case.	No record	A thin, translucent, yellow-brown, dead stripe now extends from the point of inoculation to the apex of the leaf and far down. It is about 5 mm. wide (includes 8 bundles) and 13 cm. long. The stripe is bordered by a narrow band of pale yellow tissue, beyond which, to either side and below, the leaf is a healthy green. No odor. The browning is in the bundles and the cross veinlets, the dead tissue between being pale white or a transparent yellow.	The striping has not reached the base of the leaf. Much as on the 13th, but dried out more. Leaf still green to either side. Side movement of the organism very slow.	Leaves have nearly dried out fom age, except at base.	Bulb dry and sound externally. Leaves have shriveled from age. One vascular bundle in each of 3 scales is bright yellow, broken down, and full of bacterial slime. These bundles are readily traceable into the plateau, where the diseased area widens out. Midway of the diseased scales there are yellow gum pockets surrounding the infected bundles, but the bacteria have not yet escaped into the spaces between the scales.
	Second leaf, upper part. 20 to 30 delicate needle pricks.	Pricked area pale yellow-green.	No record	Marked yellowing of the pricked area, but no death of tissue or diaphanous appearance or down striping of the disease.	Pricked part has dried out and is yellow-brown. No striping down.		

[1] The solid portion of the bulb, from which roots and scales originate.

Table showing result of inoculations with a pure culture of Pseudomonas hyacinthi (Wakker)—Continued.

No. of plant.	Date of inoculation, place, manner, and immediate result.	Result of subsequent examinations.					
	Feb. 16, 1897.	Mar. 1.	Mar. 8.	Mar. 13.	Mar. 22.	June 10.	June 23.
IV.	First leaf, upper part. Hypodermic. The injection produced a water-soaked place .5 to 1 cm. wide and 7 cm. long. In the widest places it covers 12 bundles.	Suffused water-soaked appearance over the whole injected area. By transmitted light this part of the leaf has a pale yellowish green appearance, which is decidedly different from the rest of the leaf.	No record.....	The terminal part of the leaf (7 cm.) has dried out. Below this there is a brownish stripe for a distance of 3 to 4 cm. Farther down and on either side of the stripe the leaf is a healthy green.	The narrow brown stripe has extended downward about 4 cm. farther than on the 13th. The leaf is green to either side and below.	Leaves dried out from old age, except bases.	Middle and base of bulb healthy. Two inner scales show traces of the disease in upper part (6 bundles). Barely visible to naked eye.
	Second leaf, upper part. 20 to 30 needle pricks.	Pricked area pale yellow-green.	No record.....	Marked yellowing of pricked area, but no shriveling or death of tissues and no down striping.	Pricked area yellow-brown and drying out. Narrow, yellow upward stripe (1 cm.). No down striping.		
V.	First leaf, upper part. Hypodermic. The injection produced a long, narrow, water-soaked place.	Narrow yellow stripe extending 1 cm. below point where the needle entered. No water-soaked places farther down.	No record.....	Yellow and water-soaked appearance on leaf for a breadth of 1 to 3 mm. and a length of 13 cm. The stripe now extends from the apex of the leaf halfway to the base of the leaf. In parts of the stripe there is only a very pale browning of the vascular tissue. To either side the leaf is a healthy green.	About as on 13th, but stripe dried out more. It is still narrow; color brown, with a narrow yellow margin.	Leaves dried out nearly to base (from old age).	Bulb dry and sound externally. There are 8 broken-down yellow bundles distributed in 5 different scales. In the middle of the scales two or three of the yellow bundles widen out into gum pockets, but the bacteria have not yet escaped into the spaces between the scales. The disease widens out in the base of the bulb, the plateau being badly diseased.

	Second leaf, upper part. 20 to 30 delicate needle pricks.	Pricked area pale yellow-green.	No record.....	Marked yellowing of pricked area, which begins to be transparent. No down striping.	Pricked part is drying out (yellow-brown). Two up stripes; one is narrow and brown, length 1.5 cm.; the other is wide, pale whitish-green, length 5 cm. Also two narrow brownish down stripes 1 and 1.5 cm. long.		
VI.	Leaf, upper part. Hypodermic. Two punctures, resulting in long, narrow, water-soaked places.	A very slight yellowing where the needle entered.	No record.....	A pale yellow-green stripe about 2 mm. wide, extending up 1 cm. and down the same distance. No dead tissue.	The leaf now shows a dead stripe some millimeters wide and one decimeter long.	Leaves dried out from old age nearly to base.	One bundle of one outer scale is diseased from top to base, and in the plateau the disease widens out, forming quite a good-sized gummy area. The rest of this scale and all of the others sound to naked eye (not examined microscopically).
VII.	First leaf, upper part. Hypodermic. Result, a long, narrow, water-soaked place.	A long stripe, corresponding to the injected place. Part is yellow and shriveled, while the rest looks much as it did on the day it was inoculated, i. e., watersoaked. The stripe is narrow. It extends 2 cm. above the entrance of the needle and 3 to 4 cm. below. The rest of the leaf is turgid and healthy green.	Cut away all but lower 8 cm. of leaf to photograph.	Stub of leaf healthy	No striping down of stub of leaf.	Leaves dead of old age nearly to base.	Only one bundle visibly affected (an inner scale). This is yellow and disorganized above, but the disease can not be detected by the naked eye below the middle of the bulb. Plateau sound.

Table showing result of inoculations with a pure culture of Pseudomonas hyacinthi (Wakker)—Continued.

No. of plant.	Date of inoculation, place, manner, and immediate result.	Result of subsequent examinations.					
	Feb. 16, 1897.	Mar. 1.	Mar. 8.	Mar. 13.	Mar. 22.	June 10.	June 23.
VII.	Second leaf, upper part. Hypodermic. The injection made a long, narrow, suffused streak.	Nothing very definite. Only a narrow yellow stripe 1 cm. long below entrance of needle and a small yellow spot where an abortive attempt was made to enter the needle.	No record	Stripe 1 mm. wide and 2 cm. long. Also, short yellow stripe downward from the abortive puncture.	Stripe at least 16 cm. long. Whole upper part of the leaf flabby and yellow. Leaf green below to either side of the yellow stripe and under it.		
	Third leaf, upper part. 20 to 30 ordinary needle punctures over about 1 sq. cm.	Pricked area pale yellow-green.	No record	Pricked area (12 mm. long and 6 mm. wide) is now dead, shriveled, and transparent—yellow, with pale brown veins. As yet no marked striping down.	Two narrow yellow stripes extending downward 1.5 cm. and 2.5 cm. from pricks. Disease progressing very slowly.		
VIII.	Scape. Hypodermic. Several punctures just under the unexpanded flowers.	Cut away some days since, i. e., as, soon as in full bloom. The cut was made 8 or 9 cm. under the points of inoculation and before there were any symptoms.	No record	Plant healthy.....	Plant healthy. The leaves of all of these 8 plants have doubled in size since they were inoculated.	Leaves dead nearly to base (from age).	Bulb sound externally. No results were expected, but when cut open 3 of the inner scales showed unmistakable evidence of the yellow disease. On cross section there were 6 small bright yellow spots, corresponding to as many bundles, and from these a gummy bacterial slime exuded. Four of these diseased bundles were in one scale and one in each of two others. The bacteria extended the whole length of the 6 bundles and spread out in the plateau. All the other scales were sound. (*See* Pl. I, fig. 1.)

These bulbs were planted close together in a shallow pot of sandy earth, only the lower half of each bulb being buried. The plants were on a bench in a greenhouse, where there was an abundance of light and air and where they received water from time to time as required. The external symptoms were so slight and progressed so slowly that no record was kept after March 22. The plants were, however, under almost daily observation during April and May. They made a vigorous growth for two months or more after flowering time, but as the warm weather of summer came on the leaves gradually dried out and died from the top down, and, with the exception of the bases, were pretty uniformly shriveled by the middle of June. This shriveling was not, however, the result of the disease. In fact, so little increase of symptoms was observed in April and May that when the bulbs were cut open (June 23) it was with no expectation that any diseased places would be found. That some of the leaf inoculations did dry out and fail after starting was evident, but enough succeeded to place the success of the experiment beyond doubt, one or more bundles in the bulb scales of each one of the 8 different plants being yellow, broken down, and full of bacteria (see Pl. I, fig. 1).

None of these plants became wet-rotten or bad-smelling as a result of the bacterial infection, the symptoms being wholly unlike those obtained by Dr. Heinz with his *Bacillus hyacinthi-septicus*. So far as observed the diseased plants had no odor whatever; certainly no pronounced odor. No mycelium was present in any of these yellow, broken-down bundles, or in any of the bulb scales, and in most cases no micro-organism of any sort was present except the one which had been introduced into the leaves (and scape) in the preceding February; i. e., more than four months before and at a distance of from 15 to 25 cm. from the bulbs. No animal parasites were observed.

Nearly all the scales of these eight bulbs were still entirely sound, but from the condition of the plateau, when the germs had penetrated that far, it was evident that a general infection of the scales and a more or less complete destruction of the bulbs would have been only a matter of time. Even in the attacked scales the greater part of the tissue was still sound.

Previous to making these inoculations I was inclined to attribute the slow progress of the disease in Dr. Wakker's inoculated plants to the low temperature at which his plants were kept, or else to his having used cultures containing very few living germs.[1] Having myself inoculated from a culture in prime condition for experimental purposes (i. e., swarming with motile rods), having in nearly one-half the cases inserted great numbers (that is, thousands) of the germs, hav-

[1] From my critical review, published in 1896, it will be seen that even then I was inclined to regard Dr. Wakker's statements respecting pathogenesis as substantially correct, and my subsequent studies have fully confirmed this view.

ing kept the plants at considerably higher temperatures (20° to 30° C.), and yet having obtained the same results as Dr. Wakker, I am forced to the conclusion that the organism is a rather feeble parasite and that the slowness of its progress in the plant is due to natural causes, the discussion of which I will undertake later on.

SERIES 2 (ONION).

On December 13 six shoots of an onion (*Allium cepa*) were inoculated with bright yellow slime from a potato culture (tube 12, December 4), by means of numerous needle punctures.

Result: The plant developed no leaf symptoms, and when the four bulbs (all from one root) were dug and examined in June, 1898, there was no trace of yellow bundles or other indication of disease.

NATURAL INFECTION OF A DAUGHTER BULB.

In April, 1897, a diseased bulb was potted and placed in the hothouse. This was the last remaining bulb of those received from Holland the preceding fall, the rest having been cut for study or having fallen to pieces in the dry air of the laboratory, to which they had been exposed for six months. The planted bulb did not sprout for a long time, but finally developed some feeble leaves. No particular attention was given to it during the summer and fall, but in midwinter I noticed that the leaves were dying at the top and were crooked—i. e., came up exclusively from one side of the bulb and curved over toward the other side. In February, 1898, the plant was knocked out of the pot and examined. The bulb which I had planted was completely decayed. All of the leaves were from a small daughter bulb, which was not present, or at least not visible, when the mother bulb was planted. This bulb was one-sided, had only a few leaves, and these were dying at the top. There was no wet rot of the leaves or bulb and externally the bulb was sound. On cutting it open more than forty vascular bundles in the otherwise sound white scales were found to be bright yellow, and a careful microscopic examination showed them to be full of the hyacinth germ. These yellow bundles were in eight different scales. That the daughter bulb had contracted the disease from the mother bulb which I planted was evident (1) from the fact that there was no other visible source of infection—i. e., this bulb was planted in good soil, in which hyacinths had never grown and was the only hyacinth in the greenhouse; (2) from the fact that the plateau was the most badly affected part of the bulb; and (3) from the fact that the scales seemed to have been infected from below up, the yellow slime in more than two-thirds of the affected bundles being visible to the naked eye only in the lower half of the scales, whereas in bulbs which became diseased as the result of my leaf infections the upper half of the scales (so far as examined) was always the first to show the symptoms. Probably the

leaves curved toward the decayed mother bulb from which the infection was received, as in case of one described by Dr. Wakker, but this I neglected to determine.

INOCULATIONS OF 1898.

The following year these experiments were repeated. All the plants were in the same greenhouse. The night temperature of the house for a month or two, during which symptoms were slowly extending in the hyacinth leaves, was 10° to 18° C.; the day temperature was 21° to 31° C. Subsequently, during May and June, the temperature fluctuated more, and some of the time it was considerably higher, especially in the daytime—that is, 10° to 20° C. by night and 30° to 46° C. by day. On quite a good many days during this period the air temperature for some hours ranged from 35° to 40° C.—i. e., too high for the growth of this organism, as shown by maximum temperature experiments, and probably high enough to have been of material aid to the plant in resisting the attack of the parasite.

SERIES 3 (HYACINTHS).

The third series of inoculations was made January 29. Seven well-grown hyacinth plants, not yet in bloom, were selected for this purpose, and eight uninoculated plants were held for comparison. All were inoculated from an alkaline beef-broth culture (No. 4, January 25), using a hypodermic syringe. Two were inoculated by means of numerous punctures into the short, unexpanded inflorescence. The other five plants were inoculated in the apical part of the leaves. The leaves at this time were about one-half grown (10 cm. long), and three on each plant were inoculated. Including what was wasted, about seven to eight cc. of the cloudy fluid was used on the seven plants. These plants were single-flowered and of three sorts—flowers large white with a tinge of blue, flowers large creamy white, and flowers large pink with a deeper stripe down the center of the petals; names unknown. The germs used for this series of inoculations and all of the following were descendants of those isolated in June, 1897, from the yellow, broken-down bundles in the bulbs of the plants inoculated February 16 (see first series).

Result.—Four of the check plants were destroyed by a rapid soft white rot. The other four were sound and free from all trace of the yellow disease when examined June 18.

All of the seven inoculated plants showed distinct above-ground symptoms, the progress of which was slow. Three of these were attacked in the spring by a rapid soft white rot. In one of these, which was dug early, the unsoftened part of the bulb showed two yellow bundles. The other four—i. e., those not attacked by the soft rot, were dug June 18. The bulbs were sound externally. Two were sound internally, so far as could be determined by the unaided

eye—i. e., the disease seemed to have died out in the parts above ground. In the other two there were distinct symptoms in the bulb. In one bulb several scales had yellow bundles, and the plateau was also diseased in the upper part; in the other bulb the disease was restricted to two bundles in the upper part of one scale.

SERIES 4 (ONIONS).

Three onion plants (*Allium cepa*) were selected for this series, which was begun January 29, using the same culture medium and method of inoculation as in series 3. Each plant was copiously inoculated in the apex, middle, and extreme base of several leaves.

Result.—The young and tender leaves were killed outright within a few days of the inoculation, with no distinct symptoms of parasitism. The older leaves developed no symptoms whatever, or only such as were due to the slow growth of the parasite in the immediate vicinity of the point of inoculation—i. e., the symptoms were entirely unlike those obtained by Heinz with his *Bacillus hyacinthi-septicus*. In case of half a dozen or more leaves the germ was able to hold its own in the inoculated tissues and finally to make a bright yellow growth in the parenchyma in the vicinity of the punctures. It never extended very far, however, and did not kill the parts in which it grew—at least not until after many weeks. Pl. I, fig. 2, shows the appearance of an onion leaf in which the germ has made a slow growth.

On June 22 these plants were knocked out of the pots and their bulbs examined. Each plant had a good top at this time and was in fruit. One had seven bulbs from a common root, another four, and the third three. All of these bulbs were sound. None showed any trace of yellow bundles.

SERIES 5 (HYACINTHS).

The fifth series of inoculations was made February 7, in the same way as the two preceding. These plants were inoculated from an alkaline beef broth culture (No. 1, Jan. 29), about 0.5 to 0.7 cc. of cloudy broth being used on each plant. Nine vigorous plants in full bloom were selected for this experiment, the variety being a single-flowered, pale-blue sort known as Czar Peter. Two were inoculated in the scape just under the inflorescence (0.3 cc. each, several punctures) and the remainder were inoculated in the apical portion of the leaves, three to seven leaves on each plant being selected for this purpose (generally three leaves). Twenty-three plants of the same variety and growing in the same box were held as checks.

Result.—Ten of the check plants were attacked by a soft white rot between February 7 and June 14. The bulbs of three of these were only softened a little in places when dug out and these bulbs showed no trace of yellow bundles. The other seven were destroyed by the

rot. None of the remaining thirteen check plants contracted any disease and their bulbs were sound and entirely free from yellow bundles when cut open and examined on June 14.

Two of the inoculated plants were also attacked by the same rapid soft-rot.[1] The bulb of one, which was left undisturbed, finally decayed completely; that of the other was pried out February 14 to prevent the spread of the disease. At this time there were no foliar symptoms due to the inoculations. The soft-rot had just begun. It started at the base of two leaves in wounds accidentally made by the knife of the gardener in cutting away the scape.

Distinct symptoms of the yellow disease appeared on the above-

[1] This parasite, which is a rapid-growing, bad-smelling, actively-motile white germ, probably identical with *Bacillus hyacinthi-septicus* Heinz, came from the hot house where the bulbs were forced. The box originally contained 35 bulbs, the rotten, sour-smelling remains of 3 being discovered and pried out after its purchase. All the plants inoculated in 1898 came from this same forcing house and nearly every pot or box developed some cases of this disease. Otherwise the plants were very satisfactory.

This organism was not studied critically, for lack of time, but some notes were made. The bacterial slime and accompanying tissues of the host plant taken from the upper inner part of a diseased scape (the advancing margin of the decay) were examined microscopically. There was no mycelium or insect injury, and the innumerable bacilli were apparently all one thing. The rods were 2 to 5 μ long, and rather less than 1 μ broad, with rounded ends. They were single or in pairs. Very few were in motion at first (the slime was diluted with a drop of distilled water), but within a few minutes many became actively motile. This motion consisted mostly of rapid movements straight ahead, and often straight back in the same track, for a distance many times the length of the rod. Tumbling and sinuous movements, however, were also observed. Toward the close of the first hour at least one-fourth of the rods were in motion. In form, the motile ones were exactly like the others. While watching, I frequently saw stationary rods become motile and dart away. These rods stained readily in basic fuchsin water and in gentian violet water. This slime from the host plant gave a faint bad smell and was slightly sticky, stringing up 1 centimeter. Cultures made directly into tubes of potato from the same part of this scape, after cutting it open with a burning hot knife, yielded a rather slow-growing, not very copious, wet-looking, smooth, white slime, which was strongly alkaline and somewhat sticky, stringing up 1 to 3 centimeters when touched with the loop. The four potato cultures were alike at first and three continued to be homogeneous, while a pink organism appeared in the fourth tube at the end of the second day. A few gas bubbles also appeared in each of the tubes.

This particular hyacinth plant was a robust Czar Peter in full bloom, with a long stocky scape. The rapidity of the rot may be judged from the fact that when the disease was first discovered it involved only one flower. In forty-eight hours the scape was soft-rotten (and lopped over) from the point of infection nearly to the bulb (10 or 15 centimeters) and also 3 to 5 centimeters above the point of entrance—i. e., to within a few centimeters of the top of the inflorescence. It was a soft wet rot, involving all of the tissues in a general collapse of slime which was strongly alkaline. Another fact worthy of note is that this organism is quite tolerant of acids.

That we have here a genuine bacterial disease of the hyacinth, worthy of careful study, admits of no doubt whatever.

ground parts of each of the eight other inoculated plants, and progressed slowly in the usual way. The notes upon plant No. 25, given below, will answer for all. On June 14 the bulbs of these plants were examined. One was free from bacterial infection so far as could be determined by careful cutting and microscopic examination. One was rotted and gone, as already noted. The six other bulbs were sound externally but, within, each one showed distinct symptoms of Dr. Wakker's disease—i. e., there were few to many yellow bundles full of bacteria in otherwise sound scales. In most cases the plateau was also involved. Generally the yellow disease was closely restricted to individual bundles, the parenchyma between them being sound. In several cases, however, small bacterial pockets had formed in the parenchyma around a bundle; in one case all of the parenchyma between two neighboring bundles was yellow; rarely, some of the smaller anastomosing veins would be yellowed nearly to a neighboring bundle. All of these features are shown in fig. 1 (from plant No. 20). In no case was there observed any rupture of the epidermis of the affected scales or flow of the yellow slime between the scales, the bulbs being examined too early for this stage of the disease.

FIG. 1.—Diseased scale of hyacinth.

Notes on No. 25.—Inoculated February 7 in the apical part of 5 leaves by means of a hypodermic syringe, suffused stripes resulting in each case.

February 14. Leaves 12.5 centimeters long. No symptoms. The suffused stripes due to the injection soon disappeared. The absence of symptoms is surprising, considering the quantity of germs inserted and the time that has elapsed (seven days).

March 1. Each of the five leaves now shows a yellow stripe down its center. The breadth of these stripes is 3 to 6 mm. A few of them extend from near the tip of the leaf almost to its base. Below the shorter stripes is a line of narrow, interrupted, water-soaked spots. To either side of the stripes the leaves are green and normal in appearance. On one leaf only has any tissue shriveled, and that to but a small extent. The parenchymatic tissue in the stripes has become translucent, while the parallel main bundles begin to be feebly browned. The greater part of each leaf is still healthy, the symptoms being confined to the vicinity of the injected parts. The symptoms a week ago (fourteenth day) were very slight.

April 30. A marked increase of symptoms. The stripes now extend one-half way down, two-thirds down, and entirely down to the base of the leaf. The parts which were striped on March 1 are now dead and diaphanous or brownish. The deepest brown is in the larger vascular bundles, and is feeble in comparison with the brown veining of the cabbage produced by *Ps. campestris*. At the base of these dead stripes the disease continues in the form of water-soaked stripes, which are more or less interrupted, i. e., the surface symptoms disappear and reappear a few millimeters lower. To either side of the dead, brown stripes there is a narrow yellow line beyond which the tissue is green and normal in appearance. Two of the leaves have collapsed and dried out at the tip (1 cm. and 6 cm.). The slow sidewise movement of the disease is very marked, and becomes astonishing when we consider the enormous number of germs originally inserted into these leaves. On one of these leaves there is an interrupted, water-soaked stripe in the narrow yellow border, indicating a recent slight sidewise movement of the parasites. All of these symptoms are shown in fig. 2.

June 14. The leaves are dead; the bulb is sound externally. On sectioning, the interior of the plateau was found to be diseased and there were also twenty-two yellow bundles distributed through eleven different scales. These yellow bundles were partially broken down and full of bacterial slime. That many of them were tertiary infections (from the inoculated leaves by way of the plateau) was very plain, since the yellow slime, while always distinct in the basal part of the bundle next to the plateau, frequently became less abundant or disappeared altogether in the middle or upper part of the scale. The greater part of the bulb was still sound. No mycelium was present and there were no injuries from animals.

SERIES 6 (ROMAN HYACINTHS).

Four Roman hyacinths (*Hyacinthus albulus*) were selected for this series, which was started February 7. The plants were fine specimens, in full bloom. Two of them were inoculated in the middle part of the scapes (three scapes on each plant) and the other two in the apical part of the leaves (four leaves on one plant and seven leaves on the other). The infectious material, alkaline beef-broth cultures (Nos. 1 and 4, January 29), was put in by means of a hypodermic syringe. Two plants in the same pot were held as checks.

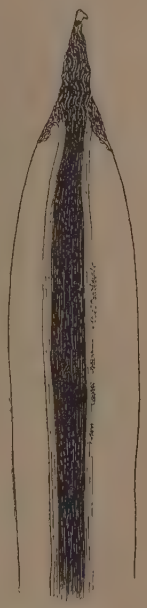

FIG. 2.—Inoculated leaf of hyacinth No. 25.

Result.—The six inoculated scapes gradually shriveled, but with no symptoms clearly attributable to the action of the germs. Each of the eleven inoculated leaves slowly developed narrow stripes corresponding to the parts of the leaf suffused at the time of the inoculation. These stripes did not appear until after the seventh day. There was very little sidewise extension, and the downward movement was very slow. At first the stripes presented a water-soaked appearance. Later they were pale yellow, with brownish veins, and when dead and dry they were yellow-brown. No such stripes appeared on any of the uninoculated leaves, of which there were many. On June 15, when all the leaves were dead and gone, the bulbs were removed from the pot and examined. Each had formed several to many daughter bulbs, but neither in these nor in the mother bulbs was there any trace of the yellow disease. All were sound so far as could be determined by the unaided eye.

SERIES 7 (HYACINTHS).

The seventh series of inoculations was made February 7, in the same manner as the preceding. For this experiment I selected sixteen vigorous plants of the single, white-flowered Baron van Tuyll, holding fifteen plants of the same variety growing in the same box as checks. The plants were in full bloom. Each bore five to seven good leaves, three of which were inoculated in the apical part. Each plant received

from 0.5 to 0.7 cc. of the cloudy alkaline beef broth culture (No. 4, Jan. 29).

Result.—Thirty-five bulbs had been planted in this box. Two were rotted and gone when it was purchased, and a rapid soft rot developed on the scapes of two others a few days later, so that only thirty-one healthy plants remained at the date of inoculation. The fifteen plants held as checks never developed any leaf symptoms comparable to those on the inoculated plants, and fourteen of the bulbs were entirely sound when dug and examined June 16. The other bulb was free from the yellow disease, but was just beginning to succumb to the soft white rot (the extreme top of the bulb).

Every one of the forty-eight inoculated leaves (sixteen plants) developed symptoms of the yellow disease. These symptoms appeared for the most part only after fifteen to thirty days, and the progress of the disease was very slow, although distinctly visible for a month or two, i. e., until the hot weather set in, when the disease seemed to die out in many leaves. On June 16, when the bulbs were dug and examined, two were soft-rotted, with the exception of a few outer scales, which showed no trace of the yellow disease. The other fourteen plants had better-preserved leaves than the corresponding plants of Czar Peter (series 5). Ten of the bulbs were entirely free from symptoms of the yellow disease and perfectly sound so far as could be determined by the unaided eye. The other four were attacked by the yellow disease, but not extensively, and for the most part only in those scales which bore the inoculated leaves. All of this variety took the disease less rapidly than the Czar Peter. The plants were very carefully examined from time to time and notes made on the condition of each one, the two sets of notes which follow being fairly illustrative of the whole lot.

Notes on plant No. 40.—Inoculated February 7 in three leaves.

February 14. No symptoms. The plant has five leaves which are now 17.5 cm. high.

March 1. One leaf only shows any decided symptoms. These consist of a stripe in the upper central part of the leaf (the inoculated part) which is yellow in the wider upper part of the stripe and water-soaked in the lower 3 to 4 cm. The length of the stripe is 9 cm., the breadth is 2 to 3 mm. in the upper part and only 1 mm. in the lower, water-soaked part. Symptoms in the other inoculated leaves are restricted to the vicinity of the needle puncture, and consist of a slight water-soaked appearance in the form of narrow, short, interrupted lines. All of this white variety have taken the disease less rapidly than the Czar Peter.

March 30. There has been a distinct progress of symptoms. On the first leaf there is a stripe of yellow-brown, dry tissue 3 mm. wide and 7 cm. long. On the second leaf there is a stripe 3 to 5 mm. wide and 3 cm. long, which is yellow with a dry, brown center. On the third leaf the stripe is 5 mm. wide and 3 cm. long. Most of this is simply yellow, but the central part is dry and brownish. Below the well-defined stripe are narrow, short, interrupted water-soaked lines on a green background. These water-soaked lines are separated from the older yellow-brown stripe by 3 to 4 cm. of healthy-looking tissue. This appearance must be due to germs which have broken out of the bundles and grown or diffused into the par-

enchyma in these particular places, or which have so destroyed the tissues as to allow the juices to flow out. The greater part of the three inoculated leaves and all of the other two leaves are sound. The leaves are now about 30 cm. long. The opinion of March 1 as to the greater resistance of this variety is certainly confirmed by the observations made to-day.

June 16. The basal 15 cm. of two of the inoculated leaves is sound. On the third it is sound, except for a narrow, interrupted, water-soaked stripe which extends to within 5 cm. of the bulb. The bulb was carefully sectioned at various levels from the base of the plateau to the top of the scales, but there was no trace of yellow bundles or any other symptom of disease. Of course it does not follow that some of these bacteria had not gained entrance to the underground parts or that six months later this bulb would not have been diseased. Indeed, I believe it would have been.

Notes on plant No. 46.—Inoculated in apical part of three leaves on Februry 7.

February 14. No symptoms. The plant has five leaves, 17.5 cm. long.

March 1. Long, narrow, water-soaked lines have appeared in the injected part of two of the inoculated leaves. As yet there is no yellowing. The third leaf shows no symptoms.

March 30. There are now distinct symptoms on each of the inoculated leaves. The stripe on the first leaf is 5 cm. long and 3 to 5 mm. wide. It is brown in the upper (widest) part, where the bulk of the injected fluid must have lodged. The tip of the second leaf is dry and brown (3 cm.), and in the middle of the leaf from this point down for a distance of 8 cm. the symptoms continue in the form of narrow, interrupted, water-soaked stripes. On the third leaf the yellow stripe is 5 mm. broad in its upper part and 1 to 2 mm. wide in the middle and lower part. Farther down the stripe is composed of narrow, interrupted, water-soaked lines on a green background. No part of the stripe is brown. The rest of the plant is normal. Here, as in No. 45, the symptoms on one leaf did not develop until after twenty-one days, and from the present appearance probably not until more than thirty days had passed. This is very remarkable, considering the number of germs used, and can be explained only on the supposition that most of them have been destroyed in the plant or, if not killed outright, have been able to overcome retarding influences only very slowly.

June 16. The basal 5 to 15 cm. of each leaf is sound externally; the rest is dead and dry. The bulb is sound externally. On cutting open, one scale only was visibly affected. This scale bore one of the inoculated leaves, and the visible symptoms were restricted to the upper third of the bulb and to one bundle. The plateau and all the other scales were free from symptoms, but probably a careful microscopic examination would have shown the beginning of disease in other bundles of this scale.

SERIES 8 (HYACINTHS).

The eighth series of inoculations was made February 11 in the same manner as the preceding. For this experiment two plants of the variety known as Gertrude were selected, and two plants of the same variety, in the same pot, were held for comparison. This variety is a deep-rose, single-flowered, vigorous-growing sort. The plants were in full bloom. One had eight leaves, the other nine. Three leaves on each plant were inoculated near the apex from the well-clouded beef-broth culture (No. 6, Feb. 5), 0.3 cc. being injected into each leaf. These leaves were 10 to 12 cm. long. The needle was inserted about 2.5 cm. from the apex of the leaf, and the narrow, suffused (water-soaked) stripe which appeared immediately after the injection of the fluid often extended nearly to the base of the leaf.

Result.—Characteristic symptoms of the yellow disease were visible on each of the inoculated leaves as early as February 14. At first the disease progressed much more rapidly than on any other variety. Later its spread was slow. The stripes in the inoculated leaves extended downward slowly until the end of March, at which date there were symptoms on no other leaves. About this time both of the inoculated plants and the two check plants were attacked and destroyed by the rapid soft white rot.

SERIES 9 (HYACINTHS).

The ninth series of inoculations was made February 11 from the same culture and in the same manner as the preceding, i. e., 0.3 cc. of the cloudy fluid was injected into each leaf. For this experiment I selected two healthy plants of a single-flowered, pale-rose variety known as Gigantea. Six plants of the same variety in the same pot were held as checks. The plants were in full bloom. Each plant had four to five leaves, three on each plant being inoculated near the apex. At this time the leaves were 9 to 11 cm. long.

Result.—On February 17 there were no symptoms on either plant. By March 1 there were pronounced stripes on four of the six inoculated leaves. The other two leaves (seventeenth day) showed no symptoms. These stripes were 2 to 3 mm. wide and 6 cm. long, extending down the middle of the leaf. The older portions of these stripes were dull yellow and semi-transparent, with pale brown bundles. Above and below this portion the striping continued in the form of water-soaked spots. To either side of these narrow stripes the leaf was healthy. The appearance of one inoculated leaf from each plant (March 5) is shown in Pl. I, figs. 3 and 4. Later, both of these plants were spoiled by the rapid soft white rot. None of the uninoculated leaves ever showed any symptoms of the yellow disease. Two of the check plants developed the rapid soft rot and were dug out soon after the experiment began. In both the rot began in the blossoms, and in one it was still confined to a single flower and a small portion of the adjacent scape when discovered. The other four check plants were dug and examined June 17. All were soft-rotted at the heart, but in the scales which remained in condition to be examined there were no yellow bundles.

SERIES 10 (HYACINTHS).

The tenth series of inoculations was made February 11 from the same culture as the preceding. For this experiment another pot of Gertrude was selected. The plants were in full bloom and very healthy. Four were inoculated and four others in the same pot were held for comparison. Each of the plants bore eight to ten leaves. Two were inoculated in the apical portion of the leaves (three leaves on each plant) by means of a hypodermic syringe, 0.3 cc. of the culture being put into each leaf. The other two were inoculated in the same way in the scape, just under the truss of flowers, several punc-

tures being made. One of these scapes received 0.3 cc. of the cloudy broth and the other 0.6 cc.

Result.—Four of the six inoculated leaves showed distinct symptoms on February 14. No stripes were visible on the other two until after February 17, and they were slight on March 1, consisting merely of some narrow, parallel, water-soaked lines. On March 1 two of the other four leaves were shriveled to the base, and a third was shriveled halfway down and showed water-soaked places farther down. Neither of the plants inoculated in the scape showed any symptoms until after February 17, all of the flowers wilting normally. On March 1 the scape which received 0.3 cc. showed one very narrow, short, water-soaked stripe in the upper part under the shriveled flowers, and at the end of this month some of the leaves began to be yellowish-green between the vascular bundles as if disturbed in their nutrition. The scape which received 0.6 cc. showed on March 1 two or three narrow, short, water-soaked lines below the shriveled flowers. At the end of this month the scape was wholly shriveled and the leaves dead at the top (upper 3 to 6 cm.). On June 17, when the bulbs were dug for examination, all were spoiled by the soft rot.

The leaves of the check plants never developed any symptoms of the yellow disease. On June 17, when the bulbs were dug for examination, all of them were soft-rotted at the heart, but none of them showed any trace of yellow bundles.

SERIES 11 (CABBAGE).

The eleventh series of inoculations was made on young cabbage plants in active growth. They were inoculated February 11 from the same culture as the preceding (No. 6, February 5). On each of two plants the germs were forced into several parts of two leaf blades by means of the syringe, and on each of the same leaf blades numerous delicate punctures were made with the tip of the needle and the fluid bearing the germs was carefully rubbed in and not allowed to dry immediately. To prevent any injurious action of sunshine or of dry air large drops of the culture were finally put on the punctured parts and sheltered from the direct action of the light and of air currents until nightfall. The germ-laden fluid was forced into 2 petioles of a third plant, so that they showed long, suffused streaks, while here and there the fluid oozed through the epidermis in many very tiny drops. The blade of a third leaf on this plant was punctured, inoculated, rubbed, covered with fluid, and sheltered as described above.

Result.—After some days the two injected petioles split open, but no other symptoms appeared, not even in the immediate vicinity of the injected and punctured parts. The plants were under observation nearly four months, and differed in no respect from the check plants. Inoculations of such plants with *Pseudomonas campestris* led to very different results, as I have shown elsewhere.[1]

[1] See Centralb. f. Bakt., 2. Abt. Bd. III, July, 1897, p. 284.

SERIES 12 (AMARYLLIS).

The twelfth series of inoculations was made February 11 on the well-grown leaves of *Amaryllis atamasco*. Two plants, not yet showing any flower shoots, were selected for this purpose, and three healthy plants in the same pot were held as checks. Two leaves were selected on each plant and 0.3 cc. of the cloudy broth culture (No. 6, February 3) was injected into each of two places on each leaf.

Result.—The symptoms developed very slowly as feeble yellowish stripes, confined to the parts originally suffused. Subsequently in the striped part the plants produced, as is their wont when injured, a red pigment. On March 1 this red pigmentation was quite distinct in each one of the eight inoculations. On March 31 it was more pronounced, occurring mostly in the form of interrupted red streaks on a green background. These were visible on one leaf for 10 cm. below one of the needle punctures and for 12 cm. above the other. On another leaf the red dots and stripes extended 7 cm. above a needle puncture and 9 cm. below it. At this point there were more than 100 red dots, each less than 0.5 mm. in diameter. These red spots were in parallel rows over vascular bundles and not in the parenchyma between the bundles. In the widest part of the stripe four vascular bundles had these red spots over them. In the oldest and worst stained part of the stripe (near the puncture) the red stain also involved the parenchyma between the bundles. After this date the disease made only very slow progress. On June 18 the bulbs were knocked out of the pot, sectioned at many levels, and carefully examined. All were entirely free from any trace of yellow bundles and perfectly sound.

SERIES 13 (HYACINTHS).

The thirteenth series of inoculations was undertaken February 12, 3 p. m., to determine whether infections might not be secured through the blossoms. For this purpose I selected four single, blue-flowered, healthy plants of Baron van Tuyll, four plants of the same variety and in the same pot being held as checks. All were in full bloom. Six flowers on each of the four plants were inoculated by putting a big drop of cloudy beef broth (No. 11, February 3) gently into the throat of each one without in any way touching the flower with the needle of the hypodermic syringe. The pot and the earth on which it stood were heavily watered and then covered with a large bell jar. This jar was removed February 14, at noon, when the drops had disappeared. Bees had access to the hothouse and visited these plants freely all day, but for the most part they carefully avoided the inoculated flowers. In one instance, however, I saw a bee enter an inoculated flower. Frequently they passed in front of such flowers and occasionally prepared to enter and then suddenly withdrew and selected uninoculated flowers.

The throat of the contracted perianth did not wet readily, and so

much uncertainty was felt as to likelihood of the infection reaching the nectaries that this series and the following one were repeated. (See series 16 and 17.)

Result.—These plants were examined March 2 and again March 31. On three of them there were no symptoms whatever and on the fourth there were symptoms of ill health, but none clearly attributable to the inoculation. (The bulb of this plant subsequently rotted.) On June 18, when these plants were next examined, one of the bulbs had soft-rotted, two appeared to be sound, and the fourth was affected with the yellow disease. My notes on this particular plant are as follows:

Notes on plant No. 63.—February 12, 3 p. m. Inoculated six flowers.

February 14, noon. Bell jar removed. The fluid has disappeared from the flowers.

February 17. The flowers are still in good condition. The inoculated ones have not shriveled or fallen off.

March 2. The scape is green and healthy to its tip. There is no evidence of any infection. The flowers have shriveled, but it is a normal withering. The leaves are sound. They are 30 cm. long and the scape is somewhat taller.

March 31. The leaves are healthy and there is no sign of yellowing, shriveling, or down-striping in the scape, which is still green and perfect to its summit.

June 18. Leaves dead, bulb sound externally except for a slight dry rot in the extreme outer part of the plateau, which entirely disappears 1 mm. in. Considerably farther up, the bulb shows distinct symptoms of the yellow disease, which increase in the plateau from below upward. In the upper part of the plateau there are quite a number of yellow bundles and several small cavities full of yellow bacteria. Near the plateau twenty-three vascular bundles in eleven scales are yellowed and more or less broken down by the bacterial slime. Farther up (near the top of the bulb) only sixteen bundles are visibly affected. These are close together on one side of the bulb in eight scales (Plate I, fig. 5). One scale of this bulb was photographed by itself and is shown in Plate I, fig. 6.

None of the check plants showed any symptoms of this disease. On June 18 three of them were entirely sound, while the fourth was partly destroyed by the soft white rot.

Infection of a daughter bulb.—On the flat side of the bulb (shown in Plate I, fig. 5), and still attached to it by a common plateau, was a good-sized daughter bulb. This was also diseased, but only where it joined the mother bulb. In the base of its plateau there were 20 vascular bundles full of the yellow slime, but the upper part of the plateau showed no symptoms and all of its scales were sound.

SERIES 14 (HYACINTHS).

This series was begun February 12 and was in all respects a duplicate of the thirteenth, except that a large single, white-flowered variety, known as Mont Blanc, was used. Six flowers on each of five plants were inoculated and three plants in the same pot were held for comparison.

Result.—Up to March 31, at which date the observations ceased, there were no symptoms on any of these plants which could be definitely ascribed to the inoculations. On June 17, when the bulbs were

dug and examined, the three check plants were entirely sound. Three of the inoculated plants were also sound, or at least appeared so to the unaided eye. The bulbs of the two other plants were sound externally, but on sectioning them they showed unmistakable symptoms of the yellow disease. One was slightly affected in two scales. The other was more seriously diseased, as will be seen from the following account of it:

Notes on plant No. 67.—February 12, 3 p. m. Inoculated six flowers.

February 14, noon. Removed the bell jar. The heart of the inoculated flowers is still moist.

February 17. The flowers begin to shrivel. The inoculated ones are holding up best.

March 2. The flowers have withered. The scape is large and 30 cm. long. Its upper 2 cm. is yellow and shriveling, but there are no symptoms attributable to the inoculations. The rest of the scape is green and turgid. The leaves are 20 cm. long and are healthy.

March 31. The scape has dry-shriveled and all of the leaves are drying out at the tip (3 to 10 cm.). The plant looks bad, but there are no stripes on the leaves, not even at their extreme base.

June 17. Leaves dead, bulb sound externally. On cutting, twenty-two yellow bundles were found in the upper part of the white and otherwise sound plateau. The infected bundles were all on one side of the bulb and were beautifully distinct, as in case of No. 63. In the upper part of the bulb eleven bundles in four scales were visibly affected, the yellow slime oozing from the cut surface. Lower down (near the plateau) a larger number of bundles were yellow, and one other scale was involved (one bundle, in which the yellow disappeared about halfway up). The extreme base of the plateau was sound, and, as in No. 63, the progress of the infection was clearly from the scape to the vessels of the plateau and from the latter to the scales. There was no soft white rot.

SERIES 15 (ONIONS).

The fifteenth series of inoculations was made February 12 on *Allium cepa*. Four well-grown onion plants not yet in bloom were selected for this purpose. The inoculations were by means of a hypodermic syringe, using the well-clouded beef broth in tube No. 11 (February 4). About 2 cc. was injected into one plant, numerous punctures being made into old and young leaves. Three leaves were selected on each of two other plants and 0.3 cc. was injected into the base of each one. The fourth plant was inoculated in the same way, 0.3 cc. being injected into the base of each of four leaves.

Result.—On March 2 the inoculated leaves, in whole or in part, were shriveled and white. On March 31 there were no additional symptoms. On June 18, when the bulbs were dug and sectioned, all were free from yellow bundles and entirely sound.

SERIES 16 (HYACINTHS).

The sixteenth series of inoculations was made February 16, at 11 a. m. Two single, white-flowered hyacinths, of the variety known as Baron van Tuyll, were selected for this purpose and two plants of the same

variety, and in the same pot, were held for comparison. The interior of eight to ten flowers on each plant was thoroughly infected by forcibly spurting 0.2 cc. of cloudy alkaline beef broth (from tube 3, February 10) into the throat of the perianth. Great care was taken not to spill any of the culture on the leaves or to wound the flowers with the tip of the needle. The pot was wet down thoroughly, covered with a bell jar, and shaded from the light. After twenty-three hours the bell jar was removed, the interior of the injected flowers being still moist. The plants were in full bloom and very thrifty.

Result.—On March 2 one scape showed a trace of water-soak in the part occupied by the flowers, but there were no additional subsequent symptoms. On June 21 these bulbs were dug and examined. Neither one showed any trace of the yellow disease. The two check bulbs were also sound. This variety took the disease slowly in series 7.

SERIES 17 (HYACINTHS).

This series was in all respects a duplicate of the preceding, except that I used two single, blue-flowered specimens of Baron van Tuyll, and inoculated a third plant in the leaves, holding two healthy plants in the same pot as checks. On February 16 eight to ten flowers were inoculated on each plant, each receiving 0.2 cc., which was spurted into the depths of the perianth, where it remained in foam. Of the plant inoculated through the leaves, one leaf received 0.4 cc. and the other two leaves 0.2 cc. each. Each foliar inoculation was made well toward the apex of the leaf.

Result.—On March 2 one of the two plants inoculated in the flowers showed distinct symptoms in the scape. These consisted of a water-soaked stripe beginning in the middle part of the inflorescence in one of the inoculated flowers. The stripe extended downward about 5 cm. and involved about one-third of the circumference of the scape. In the upper part of it the vascular bundles were feebly browned (Pl. I, fig. 7). The disease moved downward rapidly in the scape, and on March 31 the soft white rot having set in, the bulb was dug and examined. There was some yellow slime in the plateau, and one bundle of one scale was visibly invaded by the yellow microorganism. The part of the bulb recently invaded by the soft white rot was the upper central part, i. e., that previously injured by the growth of the inoculated organism. Up to March 31 the other plant developed no symptoms on the scape or leaves, but the bulb was wholly decayed when dug and examined June 17. The cause of this decay was not then determinable.

The plant inoculated through the leaves developed beautifully typical water-soaked stripes down the middle of each leaf. On March 2, two of these stripes were over 10 cm. long. On March 31 the inoculated leaves were shriveled over halfway to the bulb. This plant was not again examined until June 17, when the bulb was wholly

decayed and the cause of decay not determinable. The two check plants never developed any above-ground symptoms, and on June 17 the bulbs were entirely sound.

SERIES 18 (PLUNGE EXPERIMENT).

The eighteenth series of inoculations was made March 10, to determine whether infections could be obtained through the stomata. For this purpose I selected six pots of healthy hyacinths of the following varieties: Czar Peter, Gertrude, and Gigantea. All were in full bloom.

The material for infection consisted of 1,000 cc. of distilled water, sterilized in the ordinary way after adding 10 cc. of alkaline beef broth. When sterile, a well-developed beef-broth culture of the hyacinth germ was poured into this flask, the fluid in which was feebly clouded next morning and swarming with motile rods. On plating out, it proved to be a pure culture of the hyacinth germ. Sterile tumblers were filled with this fluid and the apical part of the leaves of selected plants were plunged into it as follows, and left twenty-three hours shaded from the light. On removal, the fluid adhering to the leaves was carefully dried in situ by exposure to the sun before the plants were left, great care being taken not to infect other parts of the same plants or of the checks.

Notes on plant No. 81.—One plant of Czar Peter, six leaves plunged 4 to 7 cm.; three healthy plants in same pot held for comparison.

March 20. No results.

March 30. Plunged part of three leaves is paler green, and one of them has a long, narrow, brown stripe. This is 13 cm. by 1 mm., and begins 0.5 cm. below the tip.

June 21. Leaves dead, bulb sound, at least to unaided vision. All of the check bulbs are free from the yellow disease and all are sound, except the outer part of one plateau, which has soft-rotted.

Notes on plant No. 82.—One plant of Czar Peter, four leaves plunged 4 to 8 cm.; three healthy plants in the same pot held for comparison.

March 30. No visible symptoms.

July 1. Bulb entirely soft-rotted. One of the check plants has also entirely soft-rotted. The other two are sound.

Notes on plant No. 83.—One plant of Gertrude, eight leaves plunged 3 to 6 cm.; seven healthy plants in the same pot held for comparison.

March 30. For the last ten days one leaf has been curved downward in the plunged part, and this part now bears alternating narrow green and yellow stripes, the latter lying in the parenchyma between the bundles. One other leaf shows slight geotropism in the plunged part and slight yellowing in stripes between the bundles. The others are normal.

July 1. The leaves are gone. The bulb has lost its center by soft rot. The scales which remain show no trace of yellow bundles. The checks were also examined. Two bulbs are sound. One is white-rotted and soft on one side, but shows no trace of the yellow germs. The other four are entirely soft-rotted and gone.

Notes on plant No. 84.—One plant of Gertrude, eight leaves plunged 3 to 5 cm.; six healthy plants in the same pot held for comparison.

March 30. No result.

June 21. Bulb rotted and gone. Of the six checks one is entirely sound, three are slightly soft-rotted, but with no trace of yellow bundles, and two have entirely decayed.

Notes on plant No. 85.—One plant of Gigantea, four leaves plunged 3 to 7 cm.; six healthy plants in same pot are held for comparison.

March 30. No symptoms.

July 1. The bulb has rotted, and it is too late to determine the cause. The bulbs of all the check plants have also rotted.

Notes on plant No. 86.—One plant of Gigantea, five leaves plunged 2 to 7 cm.; three healthy plants in the same pot were held for comparison.

March 30. No symptoms.

July 1. Leaves dead. Heart of bulb rotted out. No symptoms of the yellow disease in the scales which remain. The checks are also free from this disease. One of them has soft-rotted. The centers of the other two are also soft from the presence of the white rot.

INOCULATIONS OF 1899.

SERIES 19 (HYACINTHS).

This experiment, begun February 22, was another attempt to infect through the blossoms. From 4 to 10 flowers were inoculated by putting several small drops of the infectious fluid into the heart of the blossoms by means of a sterile hypodermic syringe. For infection, I made use of slime from an actively motile young bright-yellow culture on coconut. This slime was dissolved by shaking in a small quantity of distilled water.

The varieties tested were Regulus, blue Baron von Tuyll, white Baron von Tuyll, Gertrude, and Gigantea.

The experiment was unfortunately interrupted on June 7, at which time the bulbs of 8 of the inoculated plants were visibly affected by the yellow disease, i. e., about one-third of the whole number. About 40 plants were held as checks, none of which showed any external or internal symptoms of the disease. Regulus was affected to a greater extent than the others, but in all cases the symptoms were slight, and some months more would have been necessary for the bulbs to become seriously diseased.

REMARKS ON PATHOGENESIS.

The inoculation experiments were all made with pure cultures, on sound plants, in a hothouse where hyacinths had never before been grown, and in a country where the disease is not known to occur. Moreover, none of the several hundred check plants contracted the disease. It is therefore reasonably certain that all of the infectious material was derived from my cultures. The pathogenic nature of these cultures is rendered certain (1) because the symptoms always began in that part of the plant which was inoculated and proceeded downward, the bulb being the last part to show the disease; (2) because the organism occurring so abundantly in the yellow bundles of the bulbs was demonstrated by cultures therefrom and by microscopic examinations to be the same as that inserted into the leaves and scapes months earlier; (3) because, after cultivation on artificial

media for a year, this organism again produced the disease when inserted into the leaves and floral organs of healthy plants, and, after a lapse of some months, was again demonstrated to be present in enormous numbers in yellow broken-down bundles in the interior of the bulbs.

The time of first appearance of symptoms in the inoculated leaves varied within wide limits, according to the variety tested and the amount of material used, but nearly all the specimens of *Hyacinthus orientalis* which were inoculated showed the disease in three to thirty days in the parts above ground, and 40 of these plants also showed characteristic symptoms in the bulbs at the end of two to five months. In 1898 the conditions toward the end of the experiments were very unfavorable to the progress of the disease, owing to the extreme heat of the summer. In 1899 the experiments were disturbed and broken off too soon.

The results I have obtained indisputably confirm Dr. Wakker's statements respecting the ætiology of this disease. My studies lead me to accept substantially all of his statements regarding the character and succession of symptoms in this disease and the lesions in the host plant due to its progress. They seem to show that some varieties are more susceptible than others, e. g., Czar Peter than white Baron von Tuyll, and Gertrude than Gigantea. They show, as Wakker stated, that daughter bulbs contract the disease from mother bulbs. They do not clearly establish that the germ has any other host plant or that the parasite can enter through the stomata. They show that it is easy to induce the disease by wounds. They also indicate that bulbs may sometimes become diseased as the result of germs lodged in the flowers, and that bees sometimes visit such flowers. The last two facts point to leaf-eating and nectar-sipping insects as probable carriers of this disease. A priori, there is nothing improbable in this view, since two bacterial diseases common in the United States, the cucurbit wilt and the pear blight, are disseminated in this way, the former from germs lodged in the leaf, principally by the bites of leaf-eating beetles, the latter from germs lodged in the nectaries by bees and other insects which visit the flowers for nectar and pollen. It remains, however, for some one in the Netherlands, where the bulbs are grown in quantity, and where the disease is prevalent, to remove this statement from the domain of likelihood to that of actual fact or to show that it has no real foundation.

Wakker believed the disease to be often transmitted by the knife, and there is every reason to think his views well founded. In this case the practical deductions are easily made. Knives used on diseased plants should not be used on healthy plants until they have been thoroughly disinfected. For this purpose it is only necessary to dip them into boiling water for a few minutes.

Possibly healthy fields may become infected from the slime of the canals, into which, I am told, diseased bulbs are commonly thrown

and from which the fertile mud is raked out at stated intervals to spread over the land. From the close resemblance of this germ to *Ps. campestris*, the cause of brown rot in the cabbage, it is probable that, like the latter, the hyacinth germ is able to live for a long time in the soil of infected fields.

Diseased bulbs should be burned or put into a jar of dilute crude sulphuric acid, to which more acid is added from time to time. They should never be thrown into the canals or on waste land, nor should they be allowed to rot in place, for in this way all the soil would finally become infected. Land on which the disease is present should be used for other plants.

As suggested by Wakker, new varieties should be originated only by hand pollination, both parents being selected from such varieties as are naturally free from this disease, or which are at least little subject to it.

In concluding these remarks on pathogenesis it may be well to call special attention to certain features of this disease which seem especially instructive. The peculiarities which have impressed me most are: (1) The extremely slow progress of the symptoms—a slowness which is very remarkable if we compare it with the rapid action of such bacterial diseases as pear blight (*Bacillus amylovorus*) or the wet white rot of hyacinths which attacked some of my plants in 1898. (2) The extent to which the disease is restricted to the particular vascular bundles which are first invaded, i. e., the very slow invasion of the parenchyma and of remoter vascular bundles protected by this parenchyma.

This disease is not only peculiarly a vascular trouble, as Wakker pointed out, but is so restricted to the bundles first invaded that it seems to me impossible that there should ever be any general infection of the bulb scales until after the vessels which form a network in the plateau have become diseased. The disease was not observed in the roots.

The conditions under which this organism can grow parasitically appear to be narrowly restricted. It is not known to occur on any other host plant. It is a feeble, slow acting parasite and probably it would be confined to the domain of pure saprophytism were it not for the aeration and other peculiarly favorable conditions occurring in the vascular bundles of the hyacinth. The parenchyma of the bulb scales is distinctly acid and plainly unfavorable to its growth, most likely on account of this acidity, since studies of the organism in a variety of culture media have shown it to be peculiarly sensitive to the presence of acids, even those of the hyacinth (see Bulletin 28).

If the parenchymatic tissues of the hyacinth were less acid, if the germ were a more copious alkali producer, if it were less strictly aerobic, if it destroyed cell walls more readily, or finally, if it exerted a more powerful diastatic action on starch, it would, in my opinion, be a much more active parasite.

It is probable that a slight difference in the acidity of the parenchyma in different varieties of hyacinths is what renders some varieties more susceptible than others, but this can not be settled without further experiments which were best undertaken in the Netherlands, where according to Wakker the growers have long recognized that there are susceptible and nonsusceptible varieties.

The reader will be better able to judge of the correctness of these conclusions after reading Bulletin No. 28 in which the cultural peculiarities of this organism are discussed and compared with those of *Ps. campestris*, *Ps. phaseoli*, and *Ps. stewarti*, three other 1-flagellate, yellow bacteria common in the United States.

MORPHOLOGY OF THE PARASITE.

SIZE AND SHAPE.

This organism is a medium-sized slender rod, multiplying by fission. The ends are rounded. It is slightly variable in breadth and, under certain circumstances, greatly variable in length. Indeed, according to varying external conditions the length may be said to fluctuate enormously. Many examinations and measurements have been made. In the plant and in exhausted culture media it is generally only a single rod 1½ to 2 times as long as broad; rarely more than twice as long as broad. The appearance of some of the rods, which were taken from the daughter bulb examined in February, 1898, is shown in Plate I, fig. 8*a*. On slides stained 5 minutes in a saturated watery solution of basic fuchsin they were 0.4 to 0.5 by 0.5 to 1.0 μ. From the interior of a bulb of the first series (the slide stained June 23, 1897, in watery solution of basic fuchsin and mounted in Canada balsam and measurements made August 3, 1898), they were 0.5 by 0.9 to 1.5 μ, most of the rods on this slide being 0.5 by 1.0 to 1.2 μ. Taken from fresh cultures in beef broth they are a little longer. Plate I, fig. 8*b* shows typical forms from an alkaline beef-broth culture 9 days old. The thickest rods observed on slides made from this culture and stained in a saturated watery solution of basic fuchsin were 0.6 μ. Most of them measured 0.4 by 1.0 to 2.0 μ. On slides made the third day from a well clouded 1,000-cc. flask of distilled water containing 20 cc. of beef broth, and stained with Dr. V. A. Moore's modification of Loeffler's flagella stain, they were 0.5 to 0.7 by 1.0 to 2.0 μ. On slides made from slant agar cultures 5 days old (stock 207, acidity +22 of Fuller's scale), and stained with Alfred Fischer's flagella stain, the largest rods were 0.8 to 1.0 by 2.0 to 3.0 μ. Some of these flagella-bearing rods are shown in Plate I, figs. 9*a* and 9*b*. Flagella stains seemed to slightly increase the thickness of the rods or to render visible an outer part not stained by ordinary methods. In general the elements of this species appeared to me slenderer than those of *Ps. campestris*. Under the same conditions *Ps. phaseoli* is also a little plumper and

shorter. Flagella-bearing rods of the former are shown in Plate I, fig. 10, and of the latter in Plate I, fig. 11.

Rods were frequently seen in process of division, and occasionally two pairs were found joined end to end. Chains were never seen in the host plant or in young cultures. Even in old cultures in beef broth (rim excluded) and on potato and standard nutrient agar free from sugar, they were very rare. Prolonged search would, however, sometimes be rewarded by the discovery of a chain of 6 to 12 segments. As in case of *Ps. campestris* the tendency to form chains in the ordinary culture media is very slight. In sugar agar, on the contrary, and also on banana, sweet potato, etc., chains and long rods are very common. These are usually mixed in with zoogloeæ and the short elements. In such media the short elements often grow out into undivided filaments 50 to 150 μ in length. In many of these I was unable to discover even a trace of septa. In others the segments were distinct. Transferred to alkaline beef broth or common agar the long rods and chains disappear and the ordinary form abounds. This growth in the form of chains and filaments was observed repeatedly in cultures abounding in sugar; in fact, it may be produced at will by inoculating this organism into agar rich in grape or cane sugar. Two of these long rods taken from a 30 per cent cane sugar agar are shown in Plate I, fig. 8*c*. *Ps. campestris* and *Ps. phaseoli* behave in the same way in the presence of an excess of sugar.

No branched forms have ever been seen. Like *Ps. campestris* some of the rods appear to be slightly curved, but the chains are not crooked or twisted, as in case of vibrios.

MOTILITY.

The organism is motile, at least in early stages of its growth, in a variety of media. These movements, which are tumbling and darting, are accomplished by means of one long polar flagellum. This flagellum was stained only after repeated trials. It must be very effectually mordanted. I finally succeeded with Van Ermengem's nitrate of silver method, with Fischer's stain, and with Dr. V. A. Moore's modification of Loeffler's stain. As a rule the flagella were only feebly stained. The appearance of this organ is shown in Plate I, fig. 9. Figures of the flagella of *Ps. campestris* and *Ps. phaseoli* are introduced for comparison. In some cases it seems as if the flagellum were given off slightly below the end of the rod, both in this species and in *Ps. campestris*, but of this I could not be entirely certain. Motility was observed in potato cultures 2 to 4 weeks old, but I was never able to see any in rods taken directly from the closely packed yellow masses inside the bundles of diseased bulbs. This material was examined very carefully in distilled water.

Zooglœæ are usually developed in solid and fluid cultures after a few days, the time of appearance varying greatly with the nature of the medium. In general they appeared much sooner in acid fluids than in alkaline ones. In beef broth made very strongly alkaline to litmus (neutral to phenolphtalein) by means of caustic soda, they did not appear until the close of the second week. In acid beef broth (unneutralized) they were commonly visible to the naked eye a day or two after clouding. In one instance, however, they appeared in an alkaline gelatin culture the second day after inoculation and were very numerous the third day. This gelatin was strongly alkaline with caustic soda (neutral to phenolphtalein) and was in a fluid state (28° to 29° C.), i. e., in a condition where any substance unfavorable to growth could act on the organism most effectively. May it not be that the zooglœa stage is a protective state entered into by bacteria whenever the physical or chemical conditions of the substratum are unfavorable to growth, these conditions being either independent of the organism, as in this case, or brought about by its own metabolism?

FIG. 3.—Culture of *Pseudomonas hyacinthi* on slant 30 per cent cane-sugar agar, showing "shagreen" surface.

In beef broth and other fluid cultures the tiny aggregations of this organism showed a marked tendency to gather into a ring or rim on the wall of the tube at the level of the liquid, and sometimes floating islands appeared, but the flocculent matter seldom united into any tough pellicle, being easily jarred apart and into the depths of the fluid. These zooglœæ appear to the naked eye either as small whitish flecks or, when on the rim at the surface of the liquid, as round, yellow, colony-like bodies, especially when they have reached some age and density. These bodies also formed on substrata rich in assimilable sugars; here, perhaps, owing to the development of acids. On the solid, sugar-rich substrata, e. g., sugar-agar, potato with sugar, sugar beet, sweet potato, etc., they produced a papillose, verrucose, or shagreen-like surface, the tiny rounded elements forming this surface being very smooth and distinct in their upper part, but fused below next to the substratum. This shagreen also appeared, on old cultures, on nutrient starch jelly containing 5 per cent glycerol. This appearance is shown in figs. 3 and 4.

FIG. 4.—Slightly magnified diagrammatic views of slime of *Ps. hyacinthi* on sweet potato, showing "shagreen" surface.

SPORE FORMATION.

No spores have been seen, and I am in considerable doubt as to whether the spores observed in some of his cultures and studied so carefully by Dr. Wakker belonged to this species. He was never able to find any in the host plant, and those which appeared in his tubes may have been due to the fact that he was working at times with contaminated cultures. None of his successful infections with sporiferous material were made with spore masses entirely free from vegetative rods, and the latter are long-lived. This supposition of mixed cultures is the more likely because his work was done at a time when it was impossible to decide with ease and certainty on the purity of any given culture—i. e., before the era of poured plates—and especially because some of his gelatin cultures were certainly contaminated, i. e., yielded gas bubbles. (See Fermentation tube experiments described in Bulletin No. 28 dealing with the cultural characters of this organism.) While, therefore, not wishing to deny absolutely the existence of spores in this species, it seems to me that further and more exact proof is necessary to demonstrate their occurrence. A great many old cultures, grown on a variety of media at 18° to 26° C., have been examined without finding any spores. None were observed in the diseased bulbs, many of which were examined with care. Neither did any spores form in cultures exposed for fifteen days to air deprived of its oxygen by the potash-pyrogallic-acid method (test by microscopic examination and by exposure for ten minutes to 60° to 70° C. in alkaline beef broth). None developed in solid or fluid cultures exposed six weeks in the thermostat at 34° to 35° C. These cultures included alkaline and acid beef broth and cylinders of turnip and sugar beet standing in distilled water. Furthermore, this germ will not grow at all or grows only very feebly at the temperature which Dr. Wakker states to be most suitable for the formation of the spores viz. 35° C. (See Maximum temperature for growth, in Bulletin No. 28.) Finally, no spores developed in cultures which were first grown for a week or two at room temperatures and then put into the thermostat at 34° to 35° C. Several different media were tried, but vigorous growth stopped immediately, and after two weeks all such cultures were dead.

INVOLUTION FORMS.

Some astonishing involution forms have been observed. They formed a whitish rim at the surface of the fluid in strongly (soda) alkaline beef-broth cultures to which 10 per cent cane sugar had been added. The color was so pale that at first the tubes were supposed to be contaminated. When examined microscopically the cultures were five weeks old. These bodies were so immensely swollen, fused, twisted, and irregular in outline that seen on the slide no one to whom I showed them had any suspicion that they were bacteria. Involution forms were also seen on old turnip and banana cultures.

BEHAVIOR TOWARD STAINS.

Beyond the fact that the flagellum was stained with difficulty and that old growths, whether in the plant or out of it, took stains feebly, nothing peculiar was observed, unless it be that the bacterial precipitate resulting from growth was not stained in Dunham's solution containing methylene blue, and was stained in the same medium with rosolic acid. The following are transcripts from records scattered through my notes:

Germs from an old culture in strongly alkaline (soda) beef broth stained slowly and rather feebly in a saturated alcoholic solution of gentian violet diluted with an equal bulk of distilled water and allowed to act for half an hour. This culture had been killed by heat in the thermostat. Germs from an old culture in acid beef broth which had become alkaline, stained feebly in Ziehl's carbol fuchsin with ten minutes' exposure. This, also, was undoubtedly a dead culture. Germs from a month-old culture on sugar beet were exposed for some time to a dilute watery solution of gentian violet, whereupon all the zoogloœæ stained deeply, but the loose rods rather feebly. On long exposure (over an hour) everything stained deeply. Germs from sweet potato cultures a month old (zoogloœæ, rods, doublets, and chains) stained feebly in a deep-colored watery solution of gentian violet, although exposed for one-half hour. Germs taken from one of the bright-yellow bundles of a diseased bulb (June 23, 1897) stained feebly in water made deep red with Grübler's basic fuchsin. Germs from the yellow bundles of another bulb (Feb. 3, 1898) showed a very weak stain after five minutes' exposure to water saturated with Grübler's basic fuchsin. Exposed two minutes to water saturated with gentian violet, the stain was much better, but not deep enough. The rods from young cultures stain readily.

SYNOPSIS OF CHARACTERS.

For convenient reference I have drawn up the following brief account of this organism:

Pseudomonas hyacinthi (Wakker). A yellow, rod-shaped organism, multiplying by fission; ends rounded; single, in pairs, or 4's, more rarely in the form of chains or filaments; motile by means of one polar flagellum. In the host plant, when the bundles are crowded full of the yellow slime and broken down, it is, generally, 0.8 to 1.2 by 0.4 to 0.6 μ. In alkaline beef broth or on agar it usually measures 1.0 to 2.0 by 0.4 to 0.6 μ. In old cultures rich in sugar it often grows out into long, slender chains, or into filaments (50 to 100 μ long) in which there are no distinct septa. Nonsporiferous. Color distinctly yellow, but somewhat variable. Chrome yellow to pale cadmium in the host plant, i. e., bright yellow[1] (Ridgway's Nomenclature of Colors). On

[1] Saccardo's *flavus* and *citrinus*, but brighter (Chromotaxia). The Standard Dictionary's *yellow III*, *lemon*, and *canary*, approximately (under *Spectrum*). Prang's *yellow*, Plate I y, in the Prang Standard of Color, Popular Ed., No. 1.

culture media, when not interfered with by the brown pigment, generally gamboge, chrome yellow, or canary yellow, but sometimes paler. Old cultures on some media darken from the production of a soluble, pale-brown pigment. This feeble brown stain is best developed in hyacinth broth, in potato broth with peptone, on turnips, on radishes, and on banana rinds. It was not observed in acid or alkaline beef broth, on coconut flesh, on sugar beets, in nutrient starch jelly, in agar, or in gelatin, with or without sugar. This organism grows readily on potato cylinders standing in distilled water, but it never becomes copious or fills the water with a solid yellow slime, owing to its feeble diastatic action. Potatoes on which it has grown, even for several months, always give a strong starch reaction with iodine. It behaves the same on nutrient starch jelly free from assimilable sugars. It liquefies nutrient gelatin and Loeffler's blood serum, but does so slowly, and will not liquefy gelatin at all if 10 per cent cane sugar is added (fig. 6). Growth on nutrient agar or nutrient starch jelly is inhibited (unless the inoculation be from a solid culture and very copious) by the addition of 10 per cent glycerol, and is greatly retarded by 5 per cent glycerol; even $2\frac{1}{2}$ per cent of glycerol retarded growth. Growth in beef broth was much retarded by the addition of 1.5 per cent sodium chloride. Organism extremely sensitive to plant acids, including those of the hyacinth. Aerobic; doubtfully, if ever, facultative anaerobic; not a gas producer (see fig. 5). Does not redden litmus milk, but makes it bluer, and slowly separates the casein from the whey by means of a lab ferment. Produces under some circumstances, and slowly, a small amount of nonvolatile acid (slime acid?) with various sugars (grape, cane, etc.), which acid is frequently obscured by the moderate production of alkali. In the presence of air produces an organic acid (probably acetic) from ethyl alcohol dissolved in milk or bouillon. Inverts cane sugar, but apparently without the intervention of any enzym. Will not grow on 30 per cent grape-sugar agar. Resists dry air very well, i. e., more than forty-eight days when spread on cover glasses in thin layers.

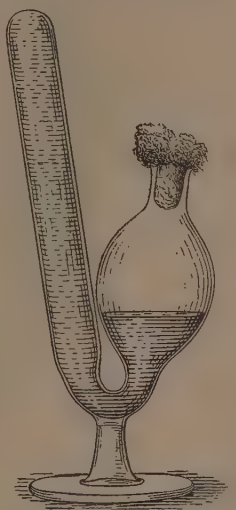

FIG. 5. Typical behavior of *Ps. hyacinthi* in fermentation tubes containing peptone water, or peptonized beef bouillon, with addition of various sugars and other carbohydrates. Fluid clear in closed end, clouded in U and open end.

In Dunham's solution with methylene blue the color is reduced in a few days, but reoxidizes quickly on shaking; final color (56 days) bright blue. In Dunham's solution with indigo carmine the color changes to a bright blue, which persists for a long time; final color yellowish. In Dunham's solution with rosolic acid and enough HCl

to render the fluid yellowish, *Ps. hyacinthi* did not redden the fluid, but made it colorless, the bacterial precipitate becoming rosy or salmon-colored. Produces indol slowly in peptonized beef broth and in peptonized Uschinsky's solution; does not produce nitrites in these solutions. Does not reduce potassium nitrate to nitrite in peptonized beef bouillon. Not a strong-smelling germ. Not readily destroyed by its own decomposition products except in media containing alcohol.

Will not grow in the thermostat at 37° C., and grows very feebly on some media and not at all on others at 34° to 35° C. Optimum temperature 28° to 30° C., or thereabouts. Minimum temperature approximately 4° C. Thermal death point (10 minutes' exposure) 47.50° C.; nearly all the rods are killed at 47° and a great many at 46.50° C. Did not grow at room temperatures after 6 days exposure in alkaline beef broth in the thermostat at 35° to 36.35°. Does not grow well in Uschinsky's solution. Grows much better in Uschinsky's solution when peptone is added to it. Grows well with a bright yellow color on cylinders of steamed coconut flesh, standing with one end in distilled water.

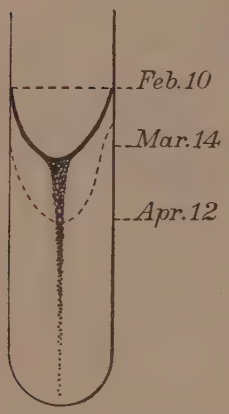

FIG. 6.—*Ps. hyacinthi* growing in strongly alkaline (0) gelatin with 10 per cent cane sugar. No liquefaction. The surface curves are due to the very gradual drying out of the gelatin.

Pathogenic to hyacinths. Enters the plant through wounds, through the blossoms, etc., and multiplies in the vascular system, filling the vessels, especially those of the bulb, with a bright yellow slime consisting of bacteria. The walls of the vessels are destroyed and extensive cavities are formed in the bundles. The parenchyma around the bundles is also involved, but only very slowly, the organism being a feeble destroyer of cell walls. The host plant is not rapidly destroyed, a year or more being necessary. The cells are first separated by solution of the middle lamella, but the wall itself seems to finally disappear. The cavities contain innumerable bacteria mingled with fragments of the dissolved bundles and of the surrounding parenchyma.

First described by Dr. J. H. Wakker from the Netherlands, where it often causes serious losses in the hyacinth gardens. Not known to occur in any other part of the world.

REMARKS ON RELATIONSHIP.

Closely related to *Ps. campestris* (parasitic on Cruciferous plants), *Ps. phaseoli* (parasitic on beans), and less so to *Ps. stewarti* (parasitic (?) on corn, especially sweet corn). Readily distinguished from the two organisms first named by (1) its brighter color; (2) its lower thermal death point; (3) its manner of growth on potato cylinders

standing in distilled water, i. e., by its feeble action on starch; and (4) its pathogenic properties. Other distinctions are given in Bulletin No. 28. Readily distinguished from *Ps. stewarti* by (1) its different, brighter color; (2) its feeble growth in Uschinsky's solution; (3) its liquefaction of gelatin and Loeffler's blood serum; (4) its lower thermal death point; (5) its lab ferment; (6) its much greater sensitiveness to acids; (7) its more luxuriant growth on turnip and rutabaga.

From facts in possession of the writer it is certain that there are many yellow organisms more or less closely related to the four mentioned in this paper, i. e., nonsporiferous, rod-shaped, micro-organisms, multiplying by fission, possessing one polar flagellum, and capable of living parasitically or semiparasitically upon various plants. All of these parasitic yellow organisms, at least all I have examined, are morphologically quite different from *Bacillus coli*, *Bacillus amylovorus*, *Bacillus tracheiphilus*, or any other micro-organism having flagella distributed over its whole surface. They also differ in many cultural peculiarities. They are, however, related to each other in many ways, and appear to form a natural group. I have an idea also that in some species the production of the brown pigment, and in others the production of the yellow pigment, has been nearly or quite extinguished. The species in which both pigments come the nearest to being equally well developed is, perhaps, *Ps. campestris*. The yellow pigment appears to be a lipochrome. (See Bul. 28.)

There are also, I believe, many morphologically similar yellow bacteria which are purely saprophytic.

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PSEUDOMONAS HYACINTHI (WAKKER) ERW. SMITH.